The Role of Genetic Diversity in Regulating the Development of Plankton-Rich Layers

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LONG-TERM GOALS

My long-term goals are focused on deciphering the role genetic diversity plays in regulating the formation and decline of plankton-rich layers (PRLs). I plan to examine 1) the mechanistic roles that genetic diversity and genetic composition play in the episodic formation of PRLs and 2) the critical temporal and spatial scales on which genetic variation influences PRL formation.

OBJECTIVES

The objective of this pilot project is to begin examining the effects of genetic diversity on the formation of plankton-rich layers. This goal will be addressed by determining the genetic composition of individual phytoplankton species (specifically diatoms) sampled from inside and outside of PRLs before, during and after their development to examine changes in genetic diversity and the effects of natural selection inside these biological hotspots. This overall objective can be divided into three goals: 1)examine the genetic composition of dominant diatom species from waters that could seed PRLs, 2)once a PRL has formed, compare genetic composition of dominant diatom species inside and outside of PRLs, 3)determine changes in genetic diversity and composition over time, as layers form and then decline.

APPROACH

Here, I am applying DNA fingerprinting techniques called microsatellites that I developed as part of a previously-funded ONR project (PI: E.V. Armbrust). Microsatellites are neutral markers that are scattered throughout the genome and consist of tandemly repeated units of DNA. The number of repeat units at a microsatellite locus can vary dramatically between individuals. Thus, the length of a repeat array is equivalent to allele size and is quantifiable as a DNA fingerprint. DNA fingerprints can then be used to 1) identify genetically distinct individuals and 2) identify genetically distinct populations, or groups of interbreeding individuals.

These techniques provide a unique tool to examine the development, persistence and decline of PRLs. For example, this pilot project aims to examine the potential importance of the "seed" population(s) present at the onset of PRL formation, the significance of intra-specific competition within PRLs and the interplay between environmental conditions and genetic composition. This project draws on

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previously developed microsatellite markers in the diatom species *Ditylum brightwellii* as well as ongoing development of markers in the diatom genus *Thalassiosira.* Because cells within a species are morphologically identical, it would normally be impossible to examine intra-species variation in the field. However, I am able to distinguish among genetically different individuals and populations using microsatellite markers and thus can examine the interplay of genetics and environment.

PRLs are sampled in East Sound, WA, a shallow, temperate fjord in the Northeastern Pacific. The fjord has a north-south extent of 9 km, an east-west width of 2 km and mean depth of 30m. Circulation is restricted by a shallow sill located at the southwestern terminus of the fjord (Fig. 1).

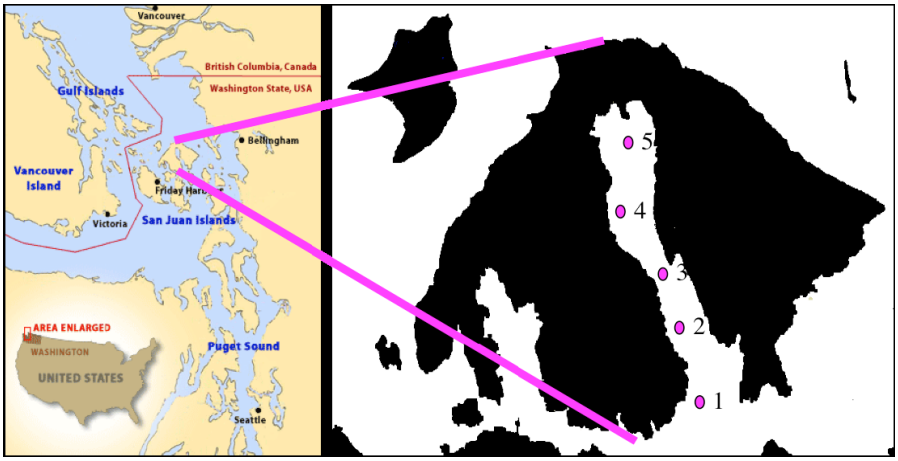

Figure 1. Location of East Sound, WA (left panel). Stations 1 and 4 will be sampled in July (pink circles, right panel). Station 1 serves as a reference station, placed just outside a sill restricting water exchange between East Sound and surrounding waters.

Sampling of PRLs is conducted in East Sound for two reasons. First, PRLs have been identified consistently inside East Sound (e.g. Cowles *et al.*, 1998, McManus et al., 2003, Menden-Deuer, in press). Second, this pilot project was conducted within the framework of an existing project led by S. Menden-Deuer (URI) and funded by the Office of Naval Research to examine PRLs in East Sound.

Sample collection, processing and statistical analyses

Briefly, ship-board measurements of chlorophyll *a* fluorescence are used to identify the depth of the PRL. Layers are sampled during the summer at intervals of 1-3 days for a total of 2-3 weeks to relate short-term changes (between days) in layer characteristics to longer-term changes (between weeks). Samples for genetic analyses are collected at station 4, inside of East Sound and station 1, outside of East Sound (Fig 1). In the laboratory, up to 50 single diatom cells are isolated from the water sample into 1ml volumes and then cultured for up to 2 weeks before harvesting for DNA extraction using well-established protocols in the Rynearson lab (Rynearson & Armbrust, 2000, Rynearson & Armbrust, 2004, Rynearson & Armbrust, 2005, Rynearson *et al.*, 2006). This pilot project focuses on two diatom genera commonly found in Washington coastal waters, *Ditylum* and *Thalassiosira* and that can be dominant in PRLs (Horner, 2002, Menden-Deuer, in press). Harvested cells are shipped to URI where genomic DNA extraction, PCR amplification of microsatellite loci and allele length determination using URI's Genomics and Sequencing facility are conducted.

Participating individuals: Laura Windecker, a graduate student at the URI Graduate School of Oceanography, was in the field to collect samples for microsatellite analyses. This field work was conducted within the framework of a larger ONR-funded field project directed by Susanne Menden-Deuer (URI, ONR award N000140710136).

WORK COMPLETED

During July and August 2007, 15 day cruises to East Sound, Orcas Island were conducted over 5 weeks. On each cruise, four stations were visited, three within East Sound and one reference station outside the sound (Fig. 1). At each station a vertical profile of the physical properties of the water column as well as phytoplankton fluorescence were recorded with a SeaBird CTD 19+ at a vertical resolution of approximately 0.2 m. A hand-held light meter (Li-Cor, LI-1400, with an underwater spherical quantum sensor SPQA 3585) was used to acquire a light profile of the water column. Real time acquisition of fluorescence data allowed us to identify phytoplankton layer presence. On all days and at all stations, water samples were collected for taxonomic analysis, size fractionated Chl a analysis (5, 10 and 20 μm) and dissolved inorganic nutrient concentrations (phosphate, nitrates and silicates). At stations 1 and 4 (outside and inside East Sound, respectively), water from at least two depths was collected for single cell isolation at Shannon Point Marine Lab.

In addition to sampling inside East Sound, we joined a cruise around the San Juan Islands and run by Shannon Point Marine Lab. This cruise allowed us to sample the genetic "background" of diatoms present in the San Juan archipelago and compare that background with the genetic identity of cells forming PRLs inside of East Sound.

RESULTS

The intensive sampling effort in East Sound, WA this past summer allows us to begin examining the relative roles of environmental variation and genetic diversity on the formation, persistence and decline of PRLs.

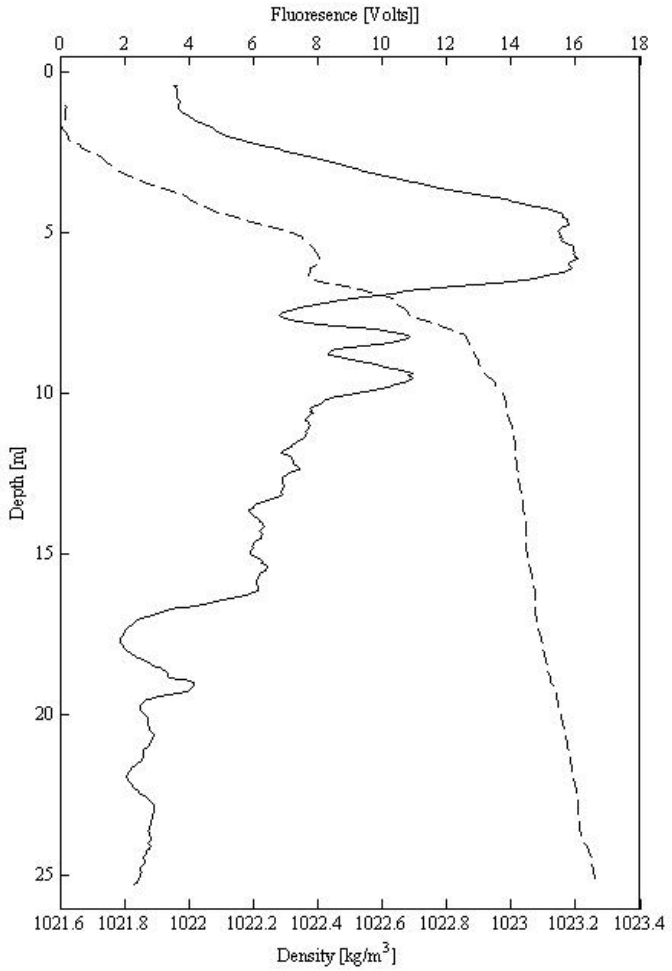

Figure 2. Depth profile of density (kg/m^3, dotted line) and fluorescence (volts, solid line) at station 4 inside East Sound on July 6, 2007. The intense peak of fluorescence at 5m depth indicates the presence of a plankton-rich layer.

Field Results: Samples were collected in changing environmental conditions over the course of 5 weeks, allowing us to observe the linkages between atmospheric conditions and biological response during layer formation, persistence and decline. Layers of high chlorophyll fluorescence were observed multiple times during the field season (see figure 2 for an example) and many of these phytoplankton layers were dominated by diatoms. During that time period, we isolated over 1000 single diatom cells from both inside and outside of layers and filtered nearly 700 of those isolates for future DNA fingerprinting (Table 1).

Table 1. Summary of single cells isolated during the 2007 field season

Summary of single cells isolated during the 2007 field season

Diatom Species	No. cells isolated	No. cells filtered for DNA fingerprinting	No. cells in culture for physiological evaluation
Ditylum brightwellii	179	96	60
Thalassiosira spp.	440	204	0
T. c.f. auguste-lineata	204	132	70
T. c.f. nordenskoldii	6	2	0
T. c.f. rotula/gravida	317	252	65
total cells	1146	686	195

In the month since field work ended, we transferred filtered cells to GSO and established nearly 200 isolates in culture at GSO for future physiological evaluation. Immediate next steps include DNA extraction of the nearly 700 filters containing single cell isolates and DNA fingerprinting of those cells.

New capabilities: This pilot project allowed us to demonstrate the feasibility of integrating genetic methods into a rigorous oceanographic field effort. A key component of this effort included developing a traveling genetic "tool box" that allows us to sample for genetic analysis side-by-side with a suite of other oceanographically relevant parameters (chl a, grazing, nutrients, physical parameters etc). The tool box includes incubators and microscopes that can shipped to remote locations for the visualization and isolation of single cells, the development of methods that permit shipping of >1000 live cells across the country for subsequent filtering and DNA analysis and the ability to sample in conjunction with another field program. This takes the newly developed molecular genetic methods from the lab bench and into the field.

IMPACT/APPLICATIONS

This pilot project has begun to examine the role of genetic diversity in regulating the formation and decline of PRLs. By sampling before and after PRL formation and inside and outside PRLs, we will begin to examine the mechanistic roles that genetic diversity and genetic composition play in the episodic formation of plankton-rich layers. Repeated sampling during the PRL from multiple locations will allow us to examine the critical temporal and spatial scales on which genetic variation influences PRL formation. Because PRLs are biological hot-spots where rates of primary production are enhanced and export production to higher trophic levels is elevated, the degree of genetic variation inside PRLs has the potential to impact diatom growth rates and thus regulate the flow of energy from the base of the marine food web up through higher trophic levels. This pilot project will provide a first glimpse into the genetic composition of diatoms inside PRLs and the implications of changes in that composition on PRL formation, persistence and decline.

RELATED PROJECTS

This pilot project was conducted within the framework of an ONR-funded project (award N000140710136) led by Dr. S Menden-Deuer (URI). The emphasis of Dr. Menden-Deuer's project is

to quantify the contribution of ecological processes to patch formation in the coastal ocean. Dr. Menden-Deuer's East Sound field project quantifies (1) spatial and temporal characteristics of large plankton patches, (2) the physical and chemical conditions these patches occur in and (3) the plankton population dynamics of the dominant layer forming species through simultaneous measurements of the biological growth and mortality rates. The rigorous field sampling included in Dr. Menden-Deuer's project will allow me to interpret the genetic data on a background of chemical, physical and biological parameters in East Sound, WA.

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